

# THE EFFECTS OF HYPERBARIC OXYGEN APPLIED FOR ONE AND FIVE DAYS ON BLOOD BRAIN BARRIER PERMEABILITY IN RATS

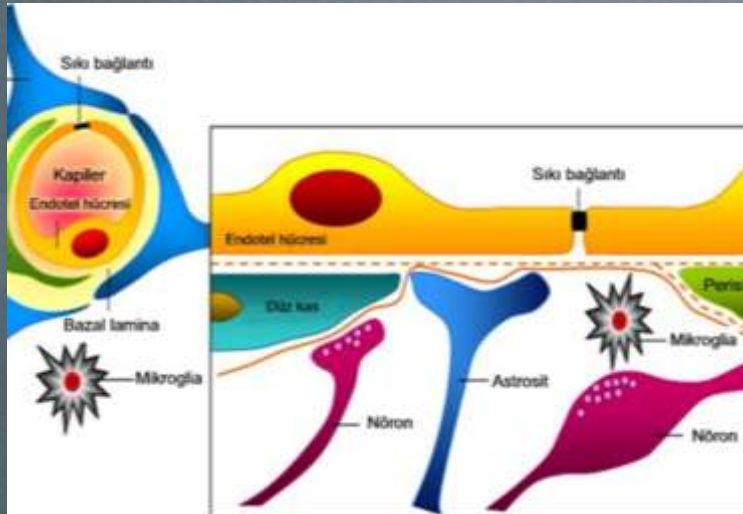
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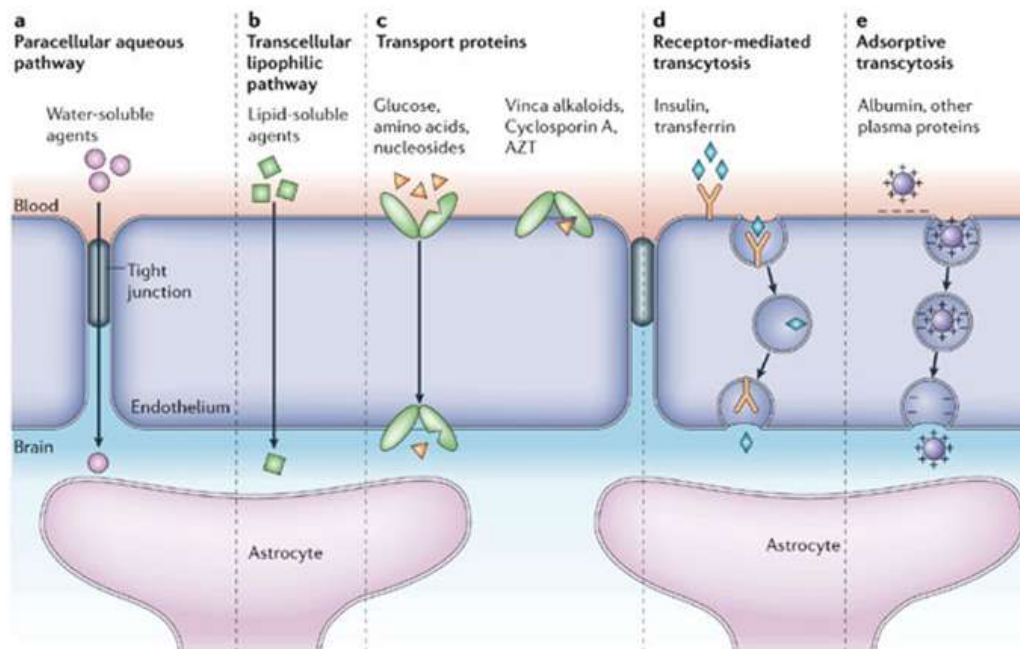
# Blood-Brain Barrier



- The blood–brain barrier is a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the central nervous system
- 1880 Paul Erlich, Trypan Blue
- 1913 Edwin Goldmann

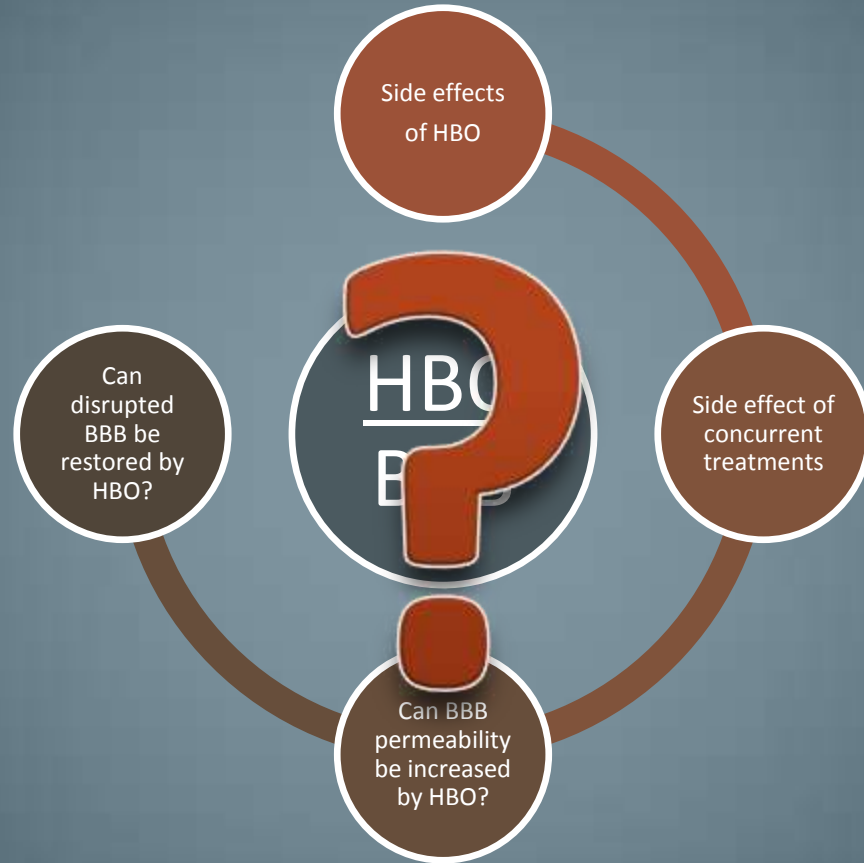


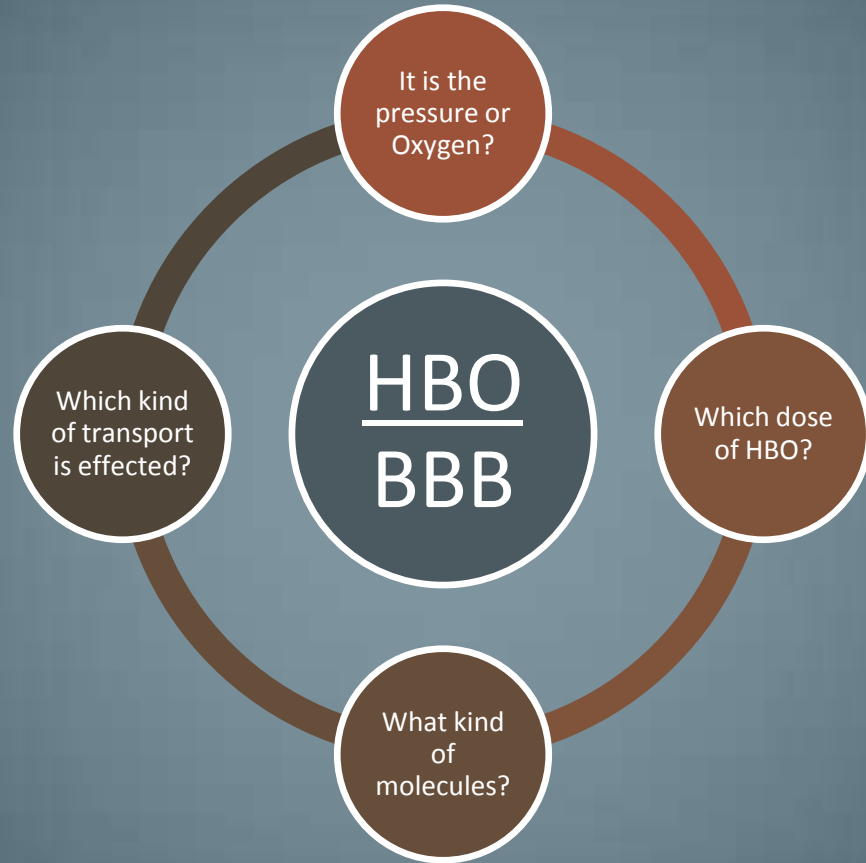
## Pathways across the blood–brain barrier



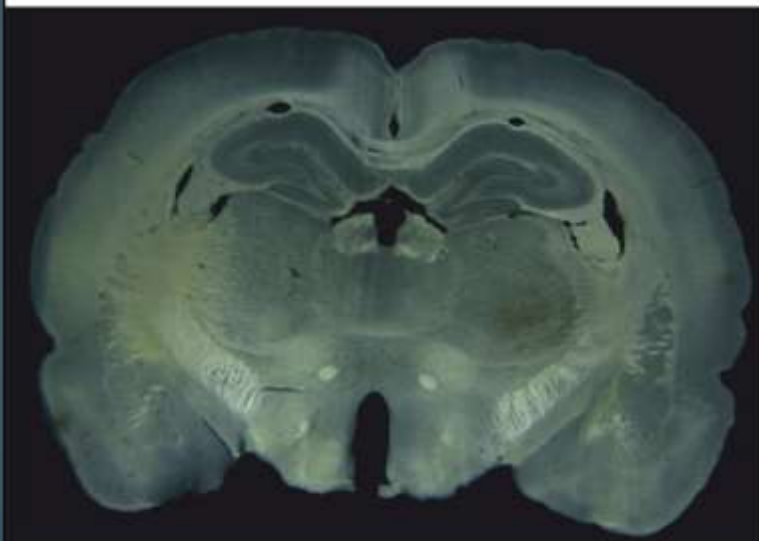
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**Nature Reviews | Neuroscience**

Abbott NJ *et al.* (2006) Astrocyte–endothelial interactions at the blood–brain barrier  
*Nat. Rev. Neuro.* **7**: 41–53 doi:10.1038/nrn1824





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## Research Report

### The effects of hyperbaric oxygen therapy on blood–brain barrier permeability in septic rats

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## ARTICLE INFO

### Article history:

Accepted 7 July 2011

Available online 18 July 2011

### Keywords:

Hyperbaric oxygen therapy

Sepsis

Blood–brain barrier

Evans blue

Homocystin peroxidase

## ABSTRACT

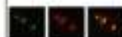
The mechanisms underlying the changes in blood–brain barrier (BBB) integrity in septic encephalopathy are poorly understood. The present study was designed to examine whether hyperbaric oxygen therapy (HBOT) influences the response of BBB to sepsis induced by cecal ligation and puncture (CLP) in rats. Cerebral cortical and hippocampal tissue levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), malondialdehyde (MDA) and glutathione (GSH) were measured. BBB permeability was functionally and structurally evaluated by determining extravasation of Evans blue (EB) and homocystin peroxidase (HSP) traces, respectively. Immunohistochemistry and western blotting for occludin were performed. HBOT did not alter TNF- $\alpha$  levels in CLP septic rats while a significant decrease was noted when the therapy was subjected to intact rats. MDA levels in animals subjected to CLP plus HBOT were significantly decreased. In septic rats, the decreased GSH levels were significantly increased by HBOT. While HBOT attenuated the increased BBB permeability to EB in rats subjected to CLP ( $P < 0.05$ ), no neurologic deficit was observed in the enhanced HSP extravasation. An increase in HSP extravasation was also observed by HBOT in intact animals. Occludin immunoreactivity and expression remained essentially unchanged in the brain capillaries of animals in all groups. Ultrastructurally, frequent vesicles containing HSP reaction products were observed in brain capillary endothelial cells of animals treated with CLP and/or HBOT. In conclusion, our results revealed that HBOT did not provide overall protective effects on the BBB integrity in septic conditions and even led to BBB disruption in intact animals.

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Abbreviations: BBB, blood–brain barrier; HBOT, hyperbaric oxygen therapy; CLP, cecal ligation and puncture; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; MDA, malondialdehyde; GSH, glutathione; EB, Evans blue; HSP, homocystin peroxidase; SE, septic encephalopathy; ROS, reactive oxygen species; TJ, tight junction; SOD, superoxide dismutase; MAPF, mean arterial blood pressure; TBSS, tile-buffered saline; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; PBS, phosphate-buffered saline.



## Research Report

## The effects of hyperbaric air and hyperbaric oxygen on blood–brain barrier integrity in rats



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## ARTICLE INFO

## Article history:

Accepted 29 July 2013

Available online 3 August 2013

## Keywords:

Hyperbaric air

Hyperbaric oxygen

Blood–brain barrier

Evans blue

Horseshoe peroxidase

Occludin

Aquaporin 4

## ABSTRACT

Hyperbaric oxygen (HBO) treatment yields conflicting results on blood–brain barrier (BBB) integrity under various pathological conditions and the effects of HBO on healthy brain is poorly understood. In this experimental study, the effects of HBO on BBB integrity were investigated in comparison with hyperbaric air (HBA) in intact rats. Four sessions of HBA or HBO were applied to intact rats in 24 h. BBB integrity was functionally and structurally evaluated by determining extravasation of Evans blue (EB) dye and horseradish peroxidase (HRP) tracers. In immunohistochemical evaluation, relative staining intensity for occludin, a tight junction (TJ) protein, and aquaporin 4 (AQP4), a water-channel protein, was detected in the barrier type of microvessels of brain by image analysis. BBB permeability to EB dye significantly increased in animals in HBO treatment group compared to those in HBA and control groups ( $p < 0.05$ ). The immunoreactivity of occludin, a tight junction protein, remained essentially unaltered in capillaries of hippocampus in all groups. In animals exposed to HBO, AQP4 immunoreactivity significantly increased in parietal cortex compared to those in HBA and control groups ( $p < 0.01$ ). Ultrastructurally, frequent vesicles containing HRP reaction products were observed in capillary endothelial cells in cerebral cortex and hippocampus of rats subjected to both HBA and HBO. Our results indicate that the HBO administration to intact rats increased BBB permeability to both EB and HRP while HBA increased only HRP extravasation in these animals. The results of this study suggest that HBA also impairs the BBB integrity in intact rats as well as HBO.





# PURPOSE

- To investigate the effect of HBO administered for 1 and 5 days on BBB barrier of the rats
- Effects on MDA, GSH and SOD

A stylized illustration of a bright yellow sun partially obscured by grey and white clouds at the top of the slide.

# MATERIAL and METHODS



HBO-1

- Oxidant/Antioxidant
- Immunohistochemistry
- Electron Microscopy

HBO-5

- Oxidant/Antioxidant
- Immunohistochemistry
- Electron Microscopy

KONTROL

- Oxidant/Antioxidant
- Immunohistochemistry
- Electron Microscopy



# MATERIAL and METHODS

- HBO
- 10' ventilation
- 30' compression/decompression
- 60' at 2,5 ATA







# MATERIAL and METHODS

- Oxidant/Antioxidant

- MDA

- GSH

- SOD



# MATERIAL and METHODS

- Immunohistochemistry
  - Glial fibrillary acidic protein (GFAP) (Glial fibrillary acidic protein is an intermediate filament (IF) protein that is expressed by numerous cell types of the central nervous system (CNS) including astrocytes and ependymal cells)

# MATERIAL and METHODS

- Electron Microscopy
  - HRP (Horseradish Peroxidaz)







# MATERIAL and METHODS

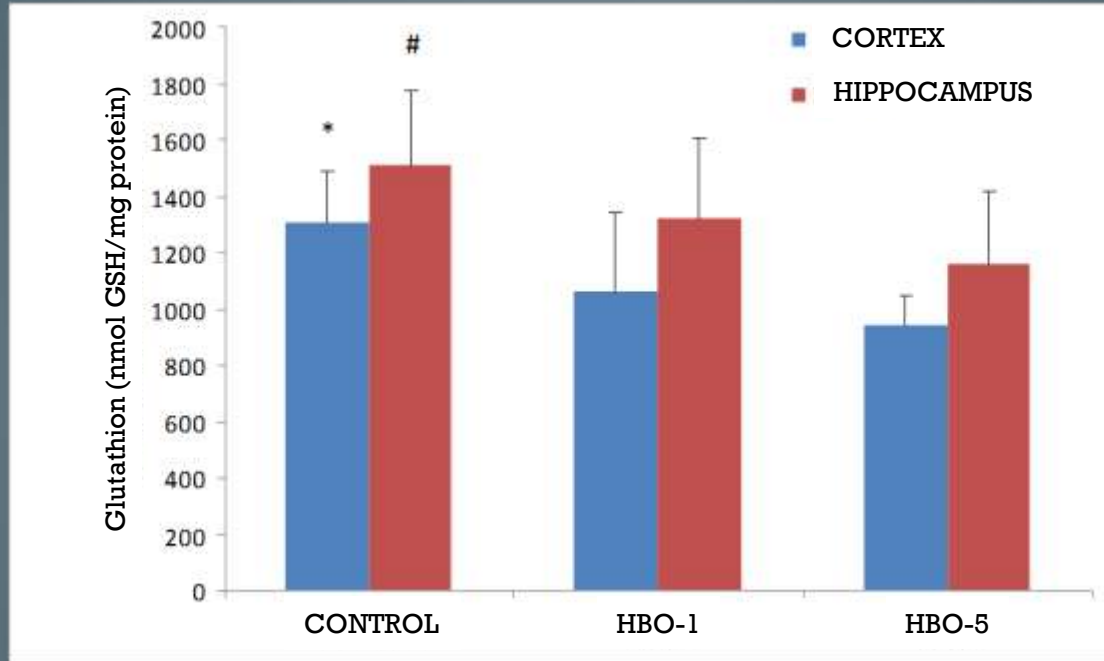
- Statistics

- SPSS 15.0

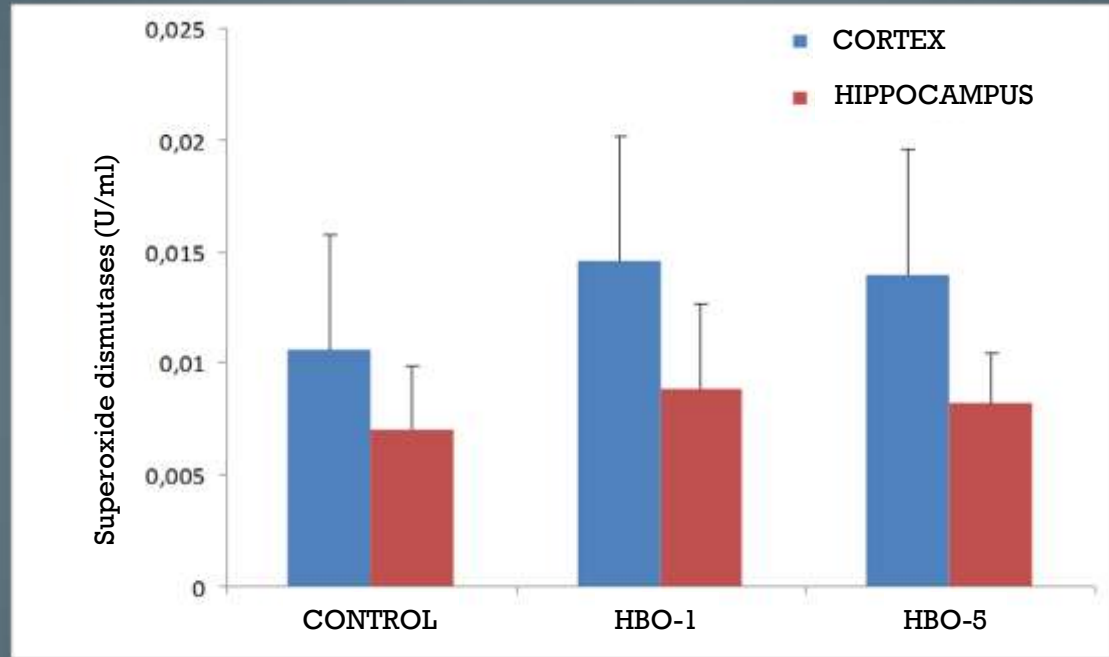
- One way ANOVA / Posthoc Tukey / Wilcoxon Signed Ranks

- $P < 0,05$

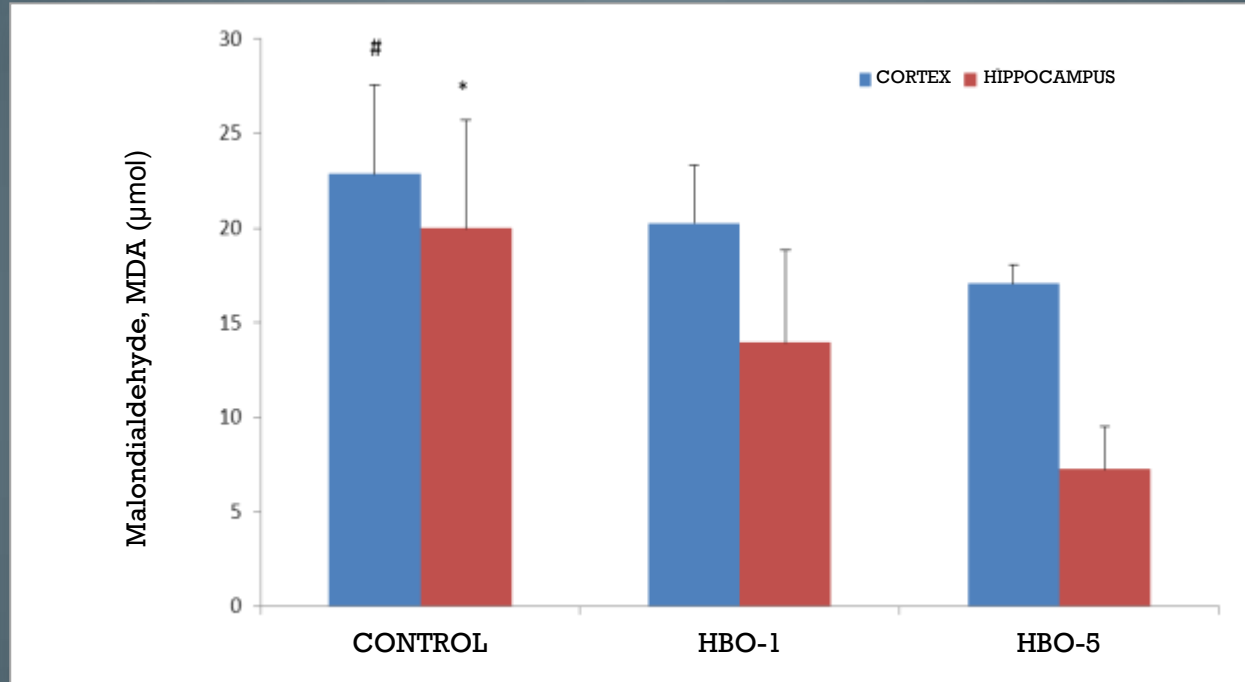




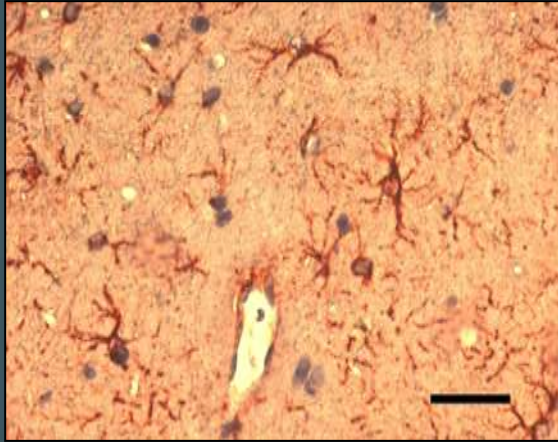
GSH levels were significantly lower in HBO-1 and HBO-5 groups



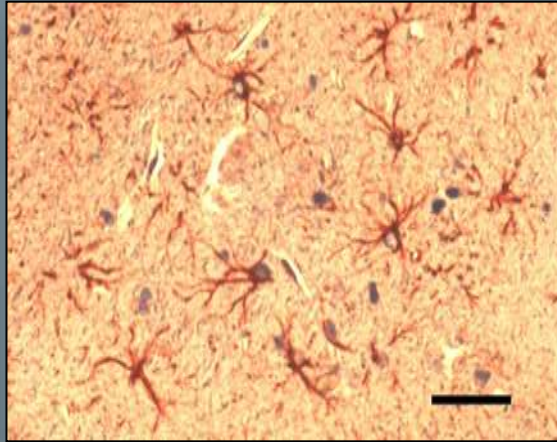
SOD levels were higher in HBO-1 and HBO-5 groups



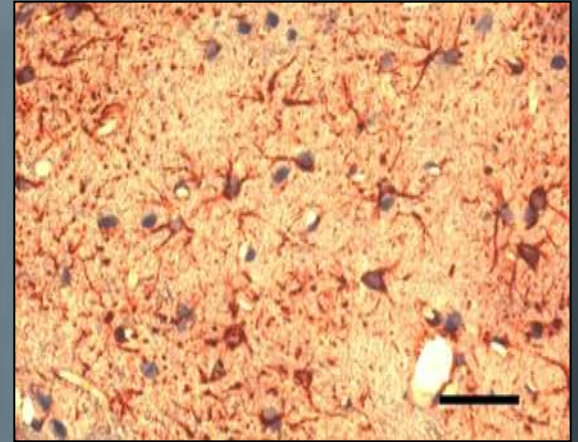
MDA levels were significantly lower in HBO-1 and HBO-5 groups, except in the cortex of the rats in HBO-1 group.



CONTROL



HBO-1



HBO-5

No significant difference in GFAP immunoreactivity was detected between groups





CONTROL

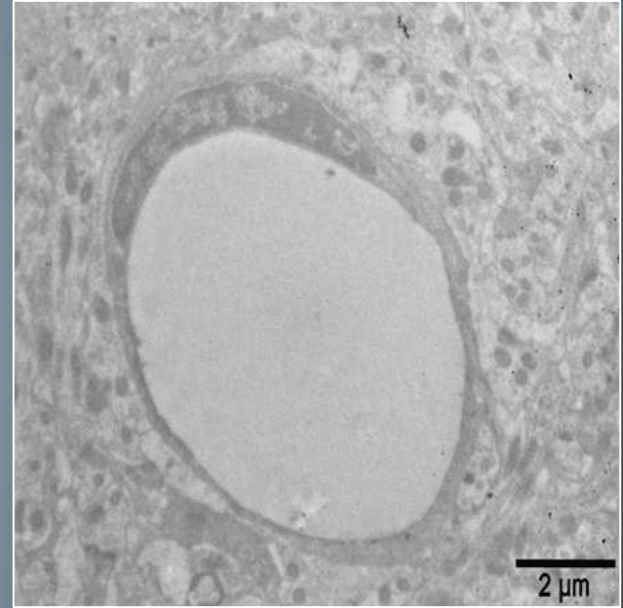
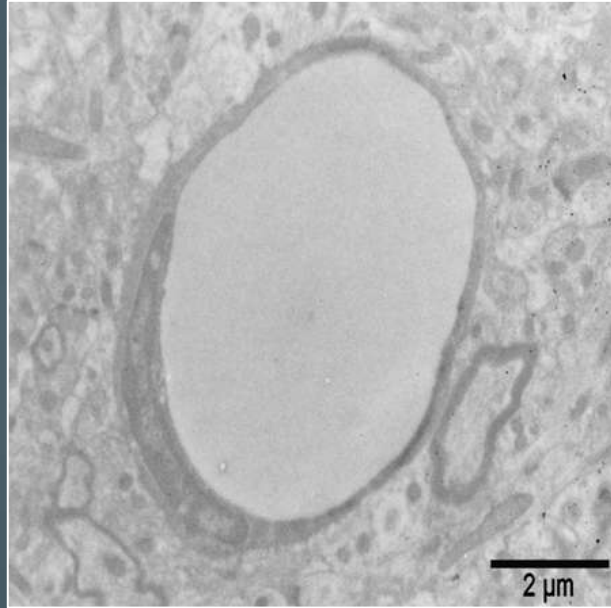


HBO-1

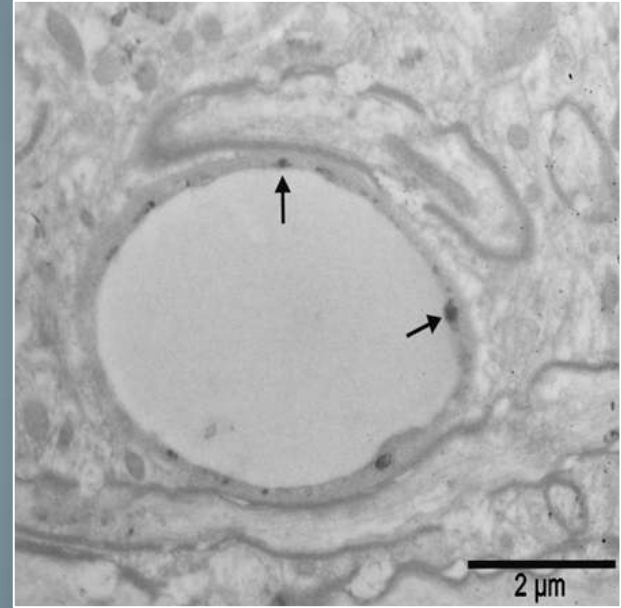
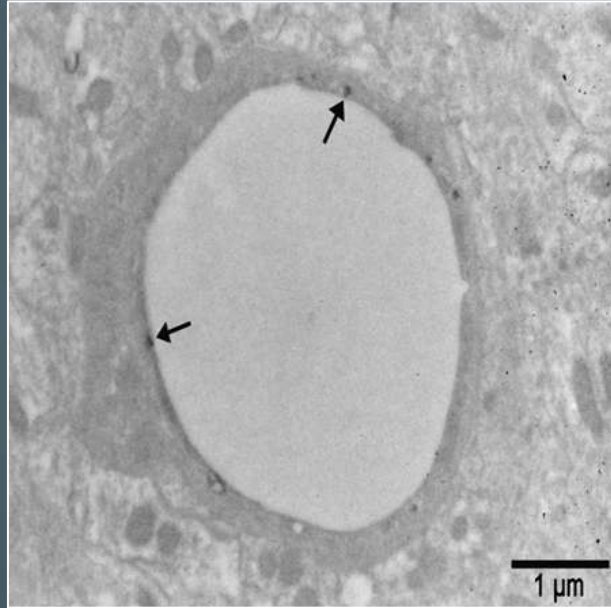


HBO-5

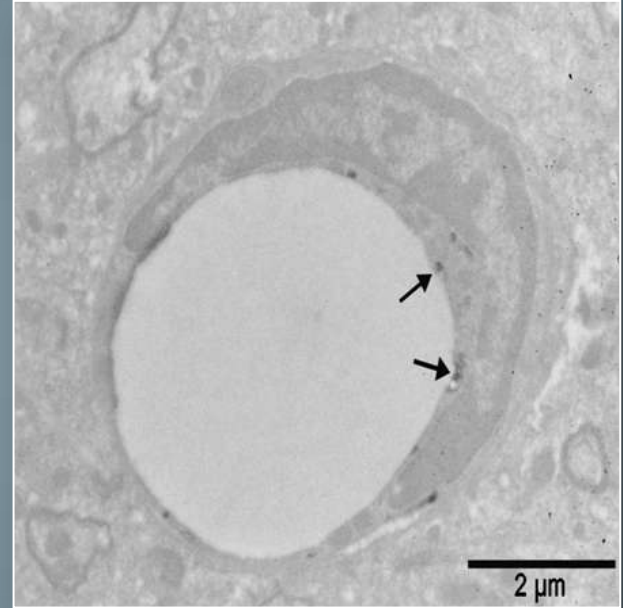
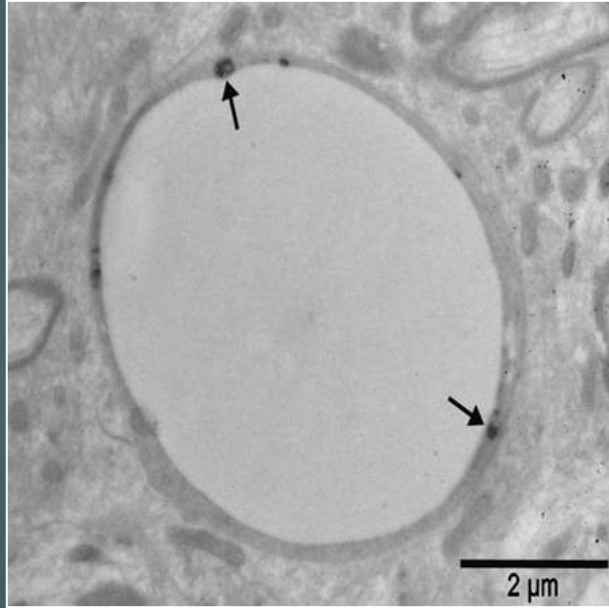
Photomicrographs showing the pattern of HRP extravasation in vibratome sections



Electron micrographs of capillaries in hippocampal and cortex regions of rats in control groups. No HRP vesicles in endothelial cells of control group



Electron micrographs of capillaries in hippocampal and cortex regions of rats in HBO-1 group. HRP vesicles in endothelial cells



Electron micrographs of capillaries in hippocampal and cortex regions of rats in HBO-5 group. HRP vesicles in endothelial cells





# CONCLUSIONS

- In the brain, MDA and GSH levels decreased, SOD activity increased by HBO
- HBO increased BBB permeability for HRP in one and five days
- In macroscopic and electron microscopic examination there was no difference in HRP reactions intensity in HBO-1 and HBO-5 groups
- Tight junctions in BBB was observed as intact by electron microscopy, therefore HRP might have passed across BBB by transcellular route



# CONCLUSIONS

- In order to clarify all effects of HBO on BBB, more studies should be done by using different doses of HBO, different hyperbaric sessions by keeping pO<sub>2</sub> fixed, different tracers with various molecular weight (probably drugs)



Thank you for your attention